Sodium Requirement for Effects of Ouabain on Contraction of Isolated Guinea Pig Atria

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SUMMARY Reducing the sodium content of medium bathing isolated guinea pig atria produces a positive inotropic response associated with a marked decline in action potential amplitude and a decrease in relaxation velocity. The effects of sodium reduction appear to result from a decline in the rate of sodium-dependent calcium efflux across the sarcolemma. Consistent with this hypothesis, caffeine, which may inhibit calcium uptake by sarcoplasmic reticulum, produces a much more pronounced inhibition of relaxation velocity in the absence than in the presence of sodium. Cardiac glycosides also appear to inhibit sodium-dependent calcium efflux, possibly by increasing intracellular free sodium. In the presence of sodium, ouabain increased developed tension in association with a decline in the velocity of relaxation. High concentrations of ouabain (>10 μM) increased resting tension in quiescent atria. In the complete absence of sodium, ouabain (100 μM) had no effect on resting or developed tension. When sodium was restored to previously sodium-free medium, resting tension declined. If atria in sodium-free medium were pretreated with ouabain or deprived of potassium, sodium restoration caused an increase in resting tension. These effects can be related to inhibition of sodium-pump activity by ouabain or potassium depletion. We conclude that both sodium reduction and cardiac glycosides increase myocardial tension development by inhibiting sodium-dependent calcium efflux across the sarcolemma. The contractile effects of ouabain are dependent completely on the presence of sodium.

SODIUM and calcium metabolism appear to be linked in cardiac muscle. Developed tension apparently is controlled primarily by the amount of calcium delivered to the contractile proteins. Luttgau and Niedergerke (1958) found that contractile strength in frog hearts did not depend solely on the extracellular concentration of calcium \([\text{Ca}^{2+}]_o\), but varied with the ratio \([\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2\). Thus, a reduction in \([\text{Na}^+]_o\) had a positive inotropic action. These authors pointed out that this relationship might be the result of a carrier in the sarcolemma which can bind and transport either two sodium ions or one calcium ion. \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange activity resembling that in cardiac muscle has been characterized extensively in squid axon (Baker et al., 1969).

According to the carrier model \([\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2 = [\text{Ca}^{2+}]_o/[\text{Na}^+]_o\), contractile strength also varies as \([\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2\) in mammalian cardiac muscle (Reuter and Seitz, 1968). In addition to accounting for the positive inotropic effect of extracellular sodium reduction, this \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange model also predicts that a positive inotropic response should result from an elevation of intracellular sodium \([\text{Na}^+]_i\).

Cardiac glycosides (>10⁻⁷ M) appear to produce sodium pump inhibition. They increase contractility, increase cellular sodium, decrease cellular potassium, and increase cellular calcium (Langer, 1972; Tillisch and Langer, 1974). It is unlikely that positive inotropy results from the outward movement of potassium, since the positive inotropic effect of cardiac glycosides can be observed during acidosis which abolishes the usual increase in potassium efflux (Poole-Wilson and Langer, 1975).

Glycosides also apparently do not affect intracellular organelles or contractile proteins since they have no influence on skinned cells (Fabiato and Fabiato, 1973). On the other hand, there is abundant evidence consistent with the hypothesis that accumulation of \([\text{Na}^+]_i\) leads to increased tension development in cardiac preparations treated with glycosides (Langer et al., 1975; Akera et al., 1977).

There is disagreement about whether the inotropic effects of cardiac glycosides can always be correlated with their ability to inhibit \(\text{Na}^+,\text{K}^+\)-ATPase activity (Allen et al., 1975; Okita, 1977; Brody and Akera, 1977). Low concentrations of ouabain (10⁻⁸ to 10⁻⁷ M) apparently produce a slight (0.02 ± 0.05 mm) decrease in \([\text{Na}^+]_o\) in some sheep Purkinje cells (as determined with sodium-sensitive microelectrodes; Ellis, 1977).

If sodium channels are blocked or inactivated in cardiac muscle, catecholamines will cause calcium-dependent action potentials or “slow responses.” Experiments on the effects of cardiac glycosides on the slow response action potential have suggested that ouabain does not enhance electrogenic inward calcium movement (Thyrum, 1974; Schneider and Sperelakis, 1974; Josephson and Sperelakis, 1977). Increasingly high (10⁻⁸ to 10⁻⁴ M) concentrations of...
ouabain were found to depress and abolish the slow response action potential. Josephson and Sperlakis have postulated that large concentrations of ouabain (>10⁻⁶ M) directly block slow channels. However, these experiments have not ruled out the possibility that slow response action potentials are compromised by some secondary effect of ouabain.

Studies of voltage-clamped cardiac preparations generally have revealed no change in inward calcium current during the early stages of glycoside exposure and a decrease in inward current after longer exposures (McDonald et al., 1975; Greenspan and Morad, 1975). However, Weingart et al. (1978) observed increases in inward calcium current in calf Purkinje fibers treated with strophanthidin, although there was a lack of parallelism between changes in slow inward current and twitch tension.

There is uncertainty regarding the mechanism(s) responsible for the inotropic and electrophysiologically effects of the cardiac glycosides. A determination of whether sodium is required for these effects would help clarify the nature of glycoside action(s). The purpose of the present study was to compare the inotropic effects of a reduction in sodium concentration and ouabain and to determine whether any contractile effects of ouabain could be observed in sodium-free media.

**Methods**

Male Hartley guinea pigs (300–500 g) were injected intraperitoneally with reserpine (5 mg/kg) 15–20 hours before they were stunned by a blow to the back of the neck. Left atria were removed and suspended in a buffer containing (in mM): NaCl, 118; KCl, 4.75; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; CaCl₂, 1; and dextrose, 5.5, maintained at 30 ± 0.5°C, pH 7.4. In some experiments, NaCl and NaHCO₃ were replaced by equiosmolar concentrations of sucrose, CaCl₂, LiCl₂, or MgCl₂. These solutions were buffered with 10 mM morpholinopropane sulfonic acid (MOPS).

Equipment for the maintenance and stimulation of isolated atria consisted of water-jacketed tissue chambers, each supplied with a gas line that delivered 95% O₂-5% CO₂ through a fine glass frit. Each atrium was hooked by two platinum-iridium wires, one of which was connected to a Statham UC2 force transducer. Atria were paced by monophasic electrical impulses 10 msec in duration. The threshold current for contractions was 1–4 mA. Some atria were stimulated with supramaximal current stimuli (40 mA). These atria were pretreated with 1 μM atropine, which blocked a negative inotropic effect, presumably mediated by electrically evoked release of endogenous acetylcholine. Diastolic tension was initially adjusted to 1.5 g; subsequently, it fell to a level between 0.5 and 1 g. The output from each force transducer was displayed on a Beckman oscillographic recorder (Dynograph). Contractions also could be monitored on a Tektronix 5111 storage oscilloscope. The voltage output from each Dynograph was converted to digital form at 1-msec intervals on an ADAC 600-LSI-11 analog-to-digital converter and stored in the memory of a DEC LSI-11 microcomputer. At 10-second intervals, these digitalized waveforms representing atrial beats were analyzed to determine diastolic tension, twitch tension (systolic-diastolic), time-to-peak tension (time from stimulation to peak tension development), and relaxation half-time (time from peak tension to half relaxation). These data were stored on a floppy disc and printed in graphic form after the experiment had been terminated. All individual records of contraction presented are typical of at least three other identically treated preparations. Statistical significance of data expressed as mean ± SEM was determined by the unpaired Student’s t-test.

**Experiments in Zero Sodium Solutions**

After an equilibration period of at least 90 minutes, some atria were transferred to medium in which NaCl and NaHCO₃ were partially or completely replaced by the corresponding choline salts supplemented with 1 μM atropine sulfate (choline is a weak muscarinic agonist). Contractions (which varied as a function of stimulus intensity) could be elicited in zero sodium media by increasing the stimulating intensity from just above the normal sodium threshold (1–4 mA) to >15 mA.

**Action Potentials**

Action potentials and contractions were recorded simultaneously from left atria immobilized in a horizontal tissue chamber and stimulated by a Grass SIU5 stimulus isolation unit. Glass microelectrodes (15–40 mΩ) were prepared from capillary tubes (1.5 mm, o.d., WPI 1B150F6) on a David Kopf Instruments model 700C vertical pipette puller and filled by boiling for 10 minutes in 3 mM KCl. Electrodes were floated on tungsten wire, 0.025 mm in diameter. Action potentials were amplified on a WPI M-707 microprobe system. Developed tension and action potentials were monitored on a Tektronix 5111 storage oscilloscope and photographed with a Tektronix C-6 oscilloscope camera.

**Sodium and Potassium Analysis**

Sodium and potassium were measured by atomic absorption spectrometry in trichloroacetic acid (10%) extracts of frozen tissues homogenized by a Polytron tissue disruptor. Before freezing, [¹⁴C]sucrose, 60,000 counts/min per ml, was added to the physiological buffer as an extracellular space marker.

**Drugs**

Reserpine (Serpasil) was obtained from Ciba Pharmaceutical. [¹⁴C]Sucrose was obtained from New England Nuclear. Atropine sulfate and oua-
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TTPT* 1 MIN 1 150 msec) twitch tension (3 g); RT 1/2 = relaxation half-time (150 msec).

**FIGURE 1** Time course of contractile effects of ouabain. A guinea pig atrium stimulated at a frequency of 0.1 Hz and an intensity of 40 mA was exposed to 1 fM ouabain at the point indicated. TTPT = time-to-peak tension (full scale = 150 msec); DIAS = diastolic tension (3 g); TWITCH = twitch tension (3 g); RT 1/2 = relaxation half-time (150 msec).

bain octahydrate were obtained from Sigma Chemical Co.

**Results**

**Dose-Dependent Effects of Ouabain on Twitch Tension and Resting Tension**

Nontoxic concentrations of ouabain (10^{-7} to 10^{-6} M) produced increases in atrial twitch (systolic-diastolic) tension associated with small decreases in the velocity of relaxation. After exposure to 1 mM ouabain, guinea pig left atria paced at 0.1 Hz developed a stable increase in developed tension (2.5- to 4-fold, n = 4) which reached a plateau in 45 minutes (Fig. 1). Diastolic tension and time-to-peak tension both increased slightly (5-7%). Relaxation half-time increased 17 ± 2%. These atria were obtained from reserpinized animals and were paced electrically with supramaximal 40-mA (10-40 times threshold) stimuli to minimize possible effects of ouabain on the release of endogenous norepinephrine,* or on the conduction velocity† of propagated action potentials. Both twitch tension and relaxation half-time increased gradually with time after the addition of ouabain.

Concentrations of ouabain in excess of 3 mM produced biphasic effects on twitch tension (Fig. 2). After an initial rise, there was a progressive decline in twitch tension associated with a gradual increase in diastolic tension, i.e., toxicity (Fig. 2, A and C). Eventually, contractions ceased and could not be restored by elevating the stimulus intensity up to 80 mA. As twitch tension steadily declined toward zero with time in the presence of high (≥10 mM) concentrations of ouabain, there was a gradual (several-fold) increase in relaxation half-time, in addition to the rise in diastolic tension. Resting tension also rose in quiescent atria exposed to high concentrations of ouabain (Fig. 2, B and D). The time course and extent of contracture were approximately the same in quiescent atria and atria paced slowly at 0.1 Hz.

**Effect of Sodium Reduction and Removal on Atrial Contractions and Resting Tension**

A reduction in extracellular sodium [Na^+]_o produced an increase in twitch tension associated with a decrease in the velocity of atrial relaxation. This result was qualitatively similar to the effect of a nontoxic concentration (1 mM) of ouabain (Fig. 1), but the time course was more rapid. The effect of a transient 50% reduction in [Na^+]_o on four parameters (computer monitored) of cardiac contractility in the same atrium paced at a frequency of 1 Hz (A) and 0.1 Hz (B) is shown in Figure 3. Oscillographic records of contraction also are displayed. At the faster frequency (1 Hz), twitch tension reached a peak 2.5 minutes after 50% reduction in [Na^+]_o and declined slightly before reaching a plateau within 10 minutes. After restoration of control

* In preliminary experiments, atria obtained from animals which were not reserpinized and exposed to 1 mM ouabain displayed variable transient decreases in time-to-peak tension and relaxation half-time and periods of automaticity. These effects were antagonized by treatment with 1 mM (-)propranolol and mimicked by the addition of 10^{-6} M (-)isoproterenol.

† Action potentials recorded from atria paced with 4-mA stimuli propagated along the tissue. There was a variable delay (0.5-10 msec) between the time of stimulation and phase 0 of the action potential, depending on the location of the microelectrode. On the contrary, depolarization of all atrial cells occurred essentially simultaneously (within 100 μsec) during 40-mA stimulation.

**FIGURE 2** Effects of toxic concentrations of ouabain on twitch tension and diastolic tension. Oscillographic records of four guinea pig atria are shown. A and C were stimulated at a frequency of 0.1 Hz and an intensity of 40 mA. B and D were quiescent. Ouabain (10 or 100 μM) was added at the points indicated.
[\text{Na}^+]_o \text{ twitch tension transiently declined below, and then returned to, the basal level. Relaxation half-time (RT 1/2) increased (25 \pm 4\%) monophasically as sodium was reduced to 50\%. Time-to-peak tension (TTPT) and diastolic tension (DIAS) were not affected greatly. When sodium was reduced in the same atrium paced at a slower rate (0.1 Hz), the results were similar, but twitch tension increased monophasically; and the rates of increase in twitch tension and relaxation half-time were slower than at the faster stimulation frequency.}

Complete sodium removal (from atria stimulated supramaximally; 40 mA) produced a biphasic effect on twitch tension associated with a large (100\%) increase in relaxation half-time (Fig. 3C). Increasing the stimulation current above 40 mA had little or no effect on twitch tension. Immediately after sodium removal, diastolic tension rose and afteroscillations§ were associated with contractions. These results are qualitatively similar to more slowly developing effects of high concentrations of ouabain (Fig. 2). Unlike ouabain (\geq 10 \mu M) which evoked increasingly severe contractures and eventual cessation of contractions, diastolic tension reached a plateau within 20 minutes after the removal of sodium (after approximately doubling), and twitches still could be evoked for several hours.

Trichloroacetic acid extracts from atria homogenized in normal sodium medium contained 1.4 \pm 0.3 \mu mol intracellular Na\(^+\) per mg protein, as determined by atomic absorption spectrometry. (The extracellular space was measured with \text{[\text{14C}\text{sucrose}]. This value was reduced 90\% after 30 minutes in 0 [\text{Na}^+], and 92\% after 60 minutes. The residual sodium may represent a tightly bound pool. After a reduction in [\text{Na}^+]_o, [\text{Na}^+]_i decreased exponentially in sheep Purkinje fibers with a half-time of 3.3 minutes (Ellis, 1977).

**Caffeine in Normal and Zero Sodium**

Caffeine (10 mM) decreased the velocity of relaxation of guinea pig atria. In the presence of a normal [\text{Na}^+]_o, caffeine increased relaxation half-time from 49 \pm 4 to 66 \pm 6 msec (or by 35\%, \(n = 4\)). Although sodium removal alone increased relaxation half-time (to 90 \pm 9 msec), 10 mM caffeine had a profound inhibitory effect on the rate of relaxation in zero sodium. Contractions in medium containing no sodium and containing 10 mM caffeine are illus-
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Effect of caffeine on atrial contractions in zero sodium. An oscillographic record of contractions elicited from a guinea pig atrium is shown. The atrium was treated with 10mM caffeine in sodium-free medium. The stimulation frequency was increased in steps as indicated. Note the very slow rate of relaxation. Similar results were observed in three other atria.

![Figure 4](http://circres.ahajournals.org/)

These data suggest that the process(es) that mediate relaxation (calcium removal) in the absence of sodium are inhibited to a large extent by 10 mM caffeine.

Lack of Effect by Ouabain on Diastolic Tension in Sodium-Free Medium

Like ouabain, sodium removal elicited an increase in resting tension in quiescent atria (Fig. 5A), with a time course similar to that in atria stimulated at 0.1 Hz (Fig. 3C). After resting tension reached a plateau in zero sodium, it could be reduced to an extent which varied inversely with $[\text{Na}^+]$, (Fig. 5B).

Ouabain had no effect on resting tension in quiescent atria equilibrated in zero sodium medium. Figure 5C illustrates the lack of effect of 100 μM ouabain on a quiescent atrium in the absence of sodium. Figure 5D shows the effect of $[\text{Na}^+]$, restoration to an atrium which had been preincubated with 100 μM ouabain for 30 minutes in the absence of sodium. The atrium was washed sequentially in ouabain-free medium containing 50%, 100%, and zero sodium. After ouabain pretreatment, return to control sodium concentration elicited only a transient decrease, followed by an increase in resting tension. Transfer from normal (100%) to zero sodium (after ouabain pretreatment) produced a transient increase in resting tension (Fig. 5D). These results can be contrasted with the results obtained without ouabain pretreatment (Fig. 5B).

Ouabain apparently bound to atria bathed in medium containing no sodium. Atria treated with 100 μM ouabain for 30 minutes in sodium-free solution, and then restored to solution with a normal sodium concentration, began developing contractions significantly ($P < 0.01, n = 4$) more rapidly than atria pretreated for 3 minutes ($52 \pm 12$ seconds vs. $191 \pm 34$ seconds). Thus, there is apparently a time-dependent binding of ouabain to a physiologically relevant receptor in sodium-free medium.

The response of atria depleted of potassium was similar to the response of atria poisoned with 100 μM ouabain. Like sodium removal and ouabain, potassium depletion would be expected to diminish sodium pump activity. Atria were bathed in sodium- and potassium-free medium for various lengths of time (Fig. 6). Total tissue potassium reached a plateau in 3–4 hours. The inset to Figure 6 shows the biphasic effect of return to control sodium concentration on resting tension in a potassium-depleted atrium. Note the similarity between this record and Figure 5D (ouabain treated). In both cases, return to control sodium concentration produced a biphasic effect on resting tension. Chronically, the addition of sodium during sodium

In preliminary experiments, total tissue potassium declined more rapidly in atria bathed in the absence of both sodium and potassium than in the absence of potassium alone. This might result from more rapid potassium loss during sodium-pump inhibition secondary to sodium removal.
pump inhibition (by potassium depletion or ouabain) produced an increase in resting tension.

Potassium-depleted atria (like ouabain-treated atria) would not contract, even in response to supramaximal stimulation. Restoration of sodium-pump activity, by the addition of potassium, produced a rapid decline in resting tension (Fig. 6) and restoration of excitability. These effects of potassium restoration were completely blocked by prior treatment with 100 JUIM ouabain (n = 4, data not shown).

Lack of Effect by Ouabain on Twitch Tension in Zero Sodium

Like resting tension, twitch tension in contracting atria suspended in sodium-free medium was completely insensitive to ouabain (100 JUIM). Figure 7 illustrates this result. A 10-min exposure to ouabain (Fig. 7A) had no effect on contractions compared to a comparably treated control (Fig. 7B). When the atria were subsequently washed in a medium containing a normal sodium concentration (not containing ouabain), evidence of toxicity rapidly became manifest in the tissue that had been exposed to ouabain. Figure 8 summarizes the results obtained in 44 atria which were treated with ouabain for 30 minutes in the presence or absence of sodium. In the presence of a normal sodium concentration, 1 JUIM ouabain produced a large increase in twitch tension with only a slight increase in diastolic tension (see also Fig. 1). Ouabain (10 JUIM) produced a severe contracture and complete cessation of beats (twitch tension = 0, see also Fig. 2). When sodium was replaced by choline, a 30-minute exposure to 1 or 10 JUIM ouabain produced no effect on twitch tension or diastolic tension. Ouabain also did not alter time-to-peak tension or diastolic tension and was without effect when sodium was replaced by sucrose, calcium, magnesium, or lithium (data not shown).

The amplitude of twitch tension varied with the stimulus intensity in sodium-free solution. This may result from abolition of the fast regenerative sodium current. The influence of stimulus intensity on tension development in the presence and absence of ouabain was examined in four atria. As illustrated in Figure 9, a 30-minute exposure to 100 JUIM ouabain failed to influence twitch tension when the stimulation intensity was varied between 16 and 40 mA.

Contractile Effects of Restoration of Control Sodium Concentration and the Influence of Ouabain

The addition of sodium to atria previously equilibrated in sodium-free medium produced biphasic effects on diastolic and twitch tension. Increasing \([Na^{+}]_o\), in increments (5, 10, 15, and 30 mm) produced an increase in twitch tension (Fig. 10A). Note that this effect is counter to that which would be expected on the basis of the \(Na^{+}-Ca^{2+}\) exchange model. Increasing \([Na^{+}]_o\), does decrease twitch tension (as expected on the basis of \(Na^{+}-Ca^{2+}\) ex-

![Figure 6](http://circres.ahajournals.org/)

**Figure 6** Potassium washout from atria. Atria were bathed in sodium- and potassium-free medium for the times indicated (with washing at 30-minute intervals). Potassium was measured in trichloroacetic acid extracts of atria by atomic absorption spectrometry. Each point represents one atrium. The inset shows the contractile effects of sodium and potassium restoration to an atrium bathed in sodium- and potassium-free medium for 4 hours.

![Figure 7](http://circres.ahajournals.org/)

**Figure 7** Effect of ouabain on contractions in individual atria bathed in sodium-free solution after sodium restoration. A illustrates the ineffectiveness of ouabain in sodium-free solution. After washing in medium containing a normal sodium concentration (not containing ouabain), an inotropic effect by ouabain became manifest. B shows a similar preparation not pretreated with ouabain. Restoration of atria to medium containing a normal sodium concentration after exposure to 100 JUIM ouabain in sodium-free solution produced a significant \((P < 0.05, n = 4)\) increase \((1.4 \pm 0.3 \text{ vs. } 0.8 \pm 0.2 \text{ (mean } \pm \text{ SEM)})\) in contractility in 10 minutes. Atria were stimulated at a frequency of 0.1 Hz and an intensity of 40 mA.
Contractile effects of ouabain require sodium/Linden and Brooker

Figure 8 Summary of the effects of ouabain on twitch tension and diastolic tension in the presence and absence of sodium. Contracting atria were exposed to 1 or 10 μM ouabain (OUA) for 30 minutes in the presence or absence of sodium. After 30 minutes of 10 μM ouabain, atria in normal sodium developed severe contractures and failed to beat (twitch tension = 0). Atria were stimulated at a frequency of 0.1 Hz and an intensity of 40 mA.

Change if sodium is elevated above 30 mM (not shown). Increasing sodium in increments from 0 to 15 mM (0, 5, 10, and 15 mM) produced a small increase in diastolic tension (Fig. 10A); as sodium was elevated from 15 to 30 mM, there was a decline in diastolic tension (Fig. 10B, A and C). The effects of restoration of control sodium concentration (to increase twitch tension and to decrease diastolic tension) were reduced and abolished if atria (in zero sodium) were pretreated for 30 minutes with 1 and 10 μM ouabain, respectively (Fig. 10B, B and C). Figure 10C illustrates how diastolic tension varies as a function of both [Na+]o and ouabain concentration. In the presence of ouabain (10 μM), addition of sodium caused diastolic tension to rise; in the absence of ouabain, addition of sodium caused diastolic tension to fall.

Effect of Sodium Removal on the Slow Action Potential

The amplitude of calcium-dependent action potentials decreased with time after sodium removal. An acute increase in twitch tension occurred despite a large decrease in the amplitude and duration of the action potential (Fig. 11, A and B). A positive inotropic response associated with a decrease in the amplitude of the slow response action potential in guinea pig atria also can be evoked by ouabain (Thyrum, 1974). Associated with a time-dependent increase in diastolic tension and a decrease in twitch tension after sodium removal (see also Fig. 3C) was a progressive decrease in the amplitude of the slow response action potential (Fig. 11, B and C). Both twitch tension and action potential amplitude plateaued after 30 minutes in zero sodium. Variations in the stimulus intensity altered both twitch tension and action potential amplitude (Fig. 11C). Since the amplitude of calcium-dependent action potentials increases with stimulus intensity, it is likely that the effect of increased stimulus intensity on twitch tension results because each cell contracts more forcefully.

Discussion

The Influence of Sodium on Cardiac Contractility

There is similarity between the effects of sodium reduction and ouabain on the contractile parameters of guinea pig atria. A nontoxic concentration of ouabain (1 μM) produced a monophasic rise in twitch tension associated with a decline in the rate of relaxation and only a slight rise in diastolic tension. Ouabain in excess of 0.1 μM produces an increase in internal sodium activity (measured with sodium-sensitive microelectrodes) in sheep Purkinje fibers (Ellis, 1977). Increasing [Na+]o by means other than adding cardiac glycosides produces a positive inotropic contractile response in guinea pig atria (Glitsch et al., 1970). The effects of a 50% reduction in [Na+]o, were similar to the effects of 1 μM ouabain; twitch tension increased in association with a decline in the velocity of relaxation. Both toxic concentrations of ouabain (10-100 μM), and complete sodium removal, produced biphasic effects on developed tension, a transient increase.
followed by a gradual decrease. The decreasing phase was associated with a rise in diastolic tension, suggesting that twitch tension declines paradoxically despite a continuing increase in intracellular free calcium during diastole. The late decline in twitch tension may be a toxic manifestation of massive intracellular calcium accumulation.

The nature of the inotropic effects associated with alterations in $[\text{Na}^+]_o$ has led several investigators to propose that the cardiac cell membrane has a means of exchanging sodium for calcium (Luttgau and Niedergerke, 1958; Reuter and Seitz, 1968; Benninger et al., 1976). Assuming a 2 for 1 exchange, $[\text{Ca}^{2+}]_i = K([\text{Na}^+]_i/[\text{Na}^+]_o)^2 [\text{Ca}^{2+}]_o$ where $K$ is a constant. The value of $K$ appears to be greater than 1 in cardiac muscle and may be influenced by membrane potential and ATP [see Benninger et al. (1976) for a detailed discussion]. If other factors are constant, the steady state level of $[\text{Ca}^{2+}]_i$ should be elevated by an increase in $[\text{Na}^+]_i$, or a decrease in $[\text{Na}^+]_o$. The ability of reduction of sodium concentration (Fig. 5) or ouabain (Fig. 2) to increase diastolic tension in quiescent tissues indicates that either of these interventions can increase...
[Ca\(^{2+}\)], without the involvement of calcium influx during the action potential. In the presence of 10 \(\mu\)M ouabain, the addition of sodium to previously sodium-free medium produced a rise in diastolic tension (Fig. 10). These data suggest that, if a sodium gradient cannot be maintained (due to the pump inhibition), the presence of sodium promotes an increase in [Ca\(^{2+}\)], possibly via Na\(^+-\)Ca\(^{2+}\) exchange. A similar conclusion can be reached based on the effect of sodium addition to potassium-depleted atria (Fig. 6).

Since both ouabain and reduction of sodium concentration decrease the velocity of relaxation (Figs. 1 and 3), the possible influence of Na\(^+-\)Ca\(^{2+}\) exchange on the rate of calcium removal during an individual contraction cycle should be considered. Twitch tension depends not only on the amount of contractile calcium delivered to the myofilaments but also on the duration of the active state. The velocity of Na\(^+-\)dependent calcium efflux during and after systole should be reduced either by an increase in [Na\(^+\)], or a decrease in [Na\(^+\)]. (assuming that Na\(^+-\)Ca\(^{2+}\) exchange contributes to this process). Two pieces of experimental information suggest that this is the case: (1) in the complete absence of sodium, relaxation half-time was increased about 100% (Fig. 3C; Linden and Brooker, 1978); (2) the gross discrepancy between the effect of 10 \(\mu\)M caffeine in sodium-containing vs. sodium-free medium (caffeine was much more effective in the absence of sodium, Fig. 4) can be resolved if one assumes two primary means for calcium removal, one involving Na\(^+-\)Ca\(^{2+}\) exchange (insensitive to caffeine) and the other involving uptake of calcium into the sarcoplasmic reticulum, which is caffeine sensitive (Thorpe, 1973). Jundt et al. (1975) demonstrated that the rate of calcium efflux from guinea pig atria in the presence of caffeine (2 \(\mu\)M) depends on [Na\(^+\)]\(_i\) as [Na\(^+\)]\(_o\) is varied between 17 and 137 \(\mu\)M.

Tillisch et al. (1979) stated that the hypothesis that Na\(^+-\)Ca\(^{2+}\) exchange contributes to relaxation is untenable since they could not measure a significant decline in \(^{45}\)Ca\(^{2+}\) efflux from the interventricular septum of rabbit unless sodium was reduced below 36 \(\mu\)M (Wendt and Langer, 1977). However, \(^{45}\)Ca\(^{2+}\) efflux studies cannot resolve the effect of sodium reduction on the velocity of calcium efflux during an individual contraction cycle—and thus the duration (and magnitude) of an individual twitch. The results of Jundt et al. (1975) and the data presented here suggest that Na\(^+-\)Ca\(^{2+}\) exchange does contribute to calcium efflux from guinea pig atria.

The Effect of Reduction of Sodium Concentration and Ouabain on Calcium Influx during the Action Potential

Sodium reduction has been reported to produce either no effect on, or to reduce the amplitude of, cardiac action potentials or calcium inward current (Brady and Woodbury, 1956; Weiss et al., 1974; Benninger et al., 1976). In the complete absence of sodium, stimuli of increasing strength produced graded electrical and mechanical responses (Fig. 11). Acutely after sodium removal, sizable twitches were associated with action potentials which were small in amplitude and duration. Similar findings were reported by Mascher (1971) in cat papillary muscle. Eyster and Reuter (1970) pointed out that in sodium-free solution even small depolarizing voltage clamps of short duration produced much larger contractions than in normal sodium solution. These data are indicative of a large contribution by nonelectrogenic calcium to contractions in sodium-free media. The source of this nonelectrogenic contractile calcium may be intracellular pools such as the sarcoplasmic reticulum (Fabiato and Fabiato, 1977). Inhibition of sodium-dependent calcium efflux may enhance the calcium content of these intracellular pools.

Declines in twitch tension after toxic ouabain doses (Fig. 2) or sodium removal (Fig. 3C) appear to be correlated with intracellular calcium accumulation, since these interventions produce a rise in diastolic tension. Afteroscillations\(^{\dagger}\) in response to ouabain or sodium removal probably also result from increased [Ca\(^{2+}\)]. (Fabiato and Fabiato, 1977). The addition of a small amount of sodium to previously sodium-free medium produced a decrease in diastolic tension and yet an increase in twitch tension (Fig. 10). These seemingly paradoxical results can be accounted for if one assumes that a very large accumulation of [Ca\(^{2+}\)] inhibits the ability of calcium to enter the myocardium during the action potential. There is a good basis for this assumption: an increase in [Ca\(^{2+}\)] decreases the duration of the action potential in cardiac muscle by increasing potassium conductance (Bassingthwaithe et al., 1976). This effect of [Ca\(^{2+}\)] on the action potential also may account for the frequency-dependent decrease in the amplitude of calcium-dependent slow action potentials observed by Weiss et al. (1974) in cat papillary muscles bathed in low sodium solutions. These authors speculated that at low frequencies, [Ca\(^{2+}\)] could be reduced to lower levels between beats and, therefore, have less of an inhibitory effect on electrogenic calcium influx.

Ellis (1977) found that a reduction in [Na\(^+\)]\(_o\), produced a decline in [Na\(^+\)]\(_o\), in sheep Purkinje fibers with a single exponential time course of 3.3 minutes. Time and frequency-dependent reequilibrations of both [Na\(^+\)]\(_o\), and [Ca\(^{2+}\)]\(_o\), after sodium reduction may contribute to the biphasic contractile effects of sodium reduction or removal (Fig. 3). The return of developed tension toward basal levels (after an initial rise) after reduction of sodium concentration was even more pronounced in rabbit intraventricular septum than in guinea pig atria (Tillisch et al., 1979).

Several investigators have found that ouabain (like reduction of sodium concentration) has no
effect on, or inhibits, the amplitude of slow calcium-dependent action potentials (Thyrum, 1974; Schneider and Sperelakis, 1974; Josephson and Sperelakis, 1977). An inhibitory effect of ouabain on calcium influx, like the effects of sodium reduction, may result as a consequence of intracellular calcium accumulation. Although 100 μM ouabain completely abolished twitches in sodium-containing medium (Fig. 2C), its lack of inhibitory effect in sodium-free solution (Figs. 7–9) indicates that ouabain does not directly inhibit slow channels as previously suggested (Josephson and Sperelakis, 1977), but possibly inhibits electrogenic calcium influx secondary to elevating [Ca²⁺].

In addition to their effects on muscle cells, cardiac glycosides may have effects on myocardial nerve terminals [see Gillis et al. (1978) for a recent review of this subject]. With the exception of Koch-Weser (1971), who observed no change in the spontaneous release of norepinephrine from isolated perfused cat heart, most investigators have reported that submicromolar doses of glycosides appear to enhance the release of endogenous catecholamines from isolated cardiac preparations (Denis et al., 1963; Tanz, 1964; Levy and Richards, 1965; Tanz and Marcus, 1966; Siefen, 1974; Harvey, 1975). Any influence that glycosides might have on the release of endogenous myocardial norepinephrine would be masked during recording of slow action potentials since they are induced by the application of exogenous catecholamines. Experiments in voltage-clamped preparations (not exposed to exogenous catecholamines) in general have verified that cardiac glycosides have no effect (initially) or decrease (chronically) inward calcium current (McDonald et al., 1975; Greenspan and Morad, 1975). However, contrary results also have been reported (Weingart et al., 1978). In this regard, it is notable that the enhanced slow inward current observed by the latter investigators in sheep Purkinje fibers in response to strophanthidin suggested “some resemblance between effects of cardiotonic steroids and sympathetic amines.” The lack of parallellism between changes in the amplitude of the slow inward current and twitch tension (also reported by these investigators) may result from a combination of direct and indirect (neural) contractile effects by the glycoside. If one considers (1) the ability of ouabain to increase diastolic tension in quiescent tissue, (2) the inability of glycosides to enhance the amplitude of slow action potentials in the presence of exogenously applied catecholamines, (3) the contradictory results obtained from voltage-clamped preparations, and (4) evidence suggesting that glycosides elicit the release of endogenous norepinephrine—it is likely that ouabain does not produce a direct effect to enhance calcium movement through slow channels. Direct effects by glycosides resulting in no effect on, or inhibition of, calcium influx during the action potential would be qualitatively similar to the effects of sodium reduction.

**Sodium Dependence of Ouabain Action**

Ouabain produced no effect at any concentration (up to 100 μM) on any contractile parameters in quiescent or electrically stimulated atria bathed in zero sodium (Figs. 5C, 6–8). Contractile responses were elicited rapidly after the return of atria exposed to ouabain to sodium-containing medium, indicating that ouabain bound to a physiologically relevant receptor in sodium-free medium. Although sodium stimulates [³H]digoxin binding to cardiac Na⁺,K⁺-ATPase, binding can occur in the absence of sodium provided that magnesium or phosphate is present (Schwartz et al., 1968). Magnesium and phosphate both were present in the media we employed. We can conclude that the complete inability of ouabain to influence contractile parameters in sodium-free media indicates that sodium is required for the inotropic actions of ouabain, and this dependence resides at some point distal to the binding of the glycoside.

Restoration of control sodium concentration to atria poisoned with ouabain (Figs. 5D and 10) caused a transient decrease followed by an increase in diastolic tension. This can be viewed as resulting from a transient efflux of [Ca²⁺], in exchange for added [Na⁺]. Since the tissue could not maintain a sodium gradient, sodium (and calcium via Na⁺-Ca²⁺ exchange) leaked into cardiac cells and gradually produced a rise in resting tension. The subsequent reduction in [Na⁺] produced a transient efflux of intracellular Na⁺ in exchange for extracellular Ca²⁺ and a transient increase in resting tension (Fig. 5D). A pronounced transient increase in resting tension did not result when sodium was removed from an atrium not treated with ouabain (Fig. 5B), presumably because [Na⁺] was maintained at a low level if Na-pump activity was not inhibited. After ouabain poisoning, resting tension appears to act like a calcium-sensitive “electrode,” and alterations in [Na⁺], produce changes in resting tension which would be expected on the basis of the Na⁺-Ca²⁺ exchange model. A prolonged exposure to potassium-free medium produced an analogous situation in which potassium-lack rather than ouabain was used as a means to inhibit sodium-pump activity (Fig. 6).

Several investigators have suggested that sodium pump inhibition may not be required for the positive inotropic action of cardiac glycosides. Studies attempting to correlate inotropic effects of glycosides with inhibition of Na⁺,K⁺-ATPase activity are difficult to interpret due to problems associated with removal of the enzyme from intact cells and contradictory results (Allen et al., 1975; Okita, 1977; Brody and Akerblom, 1977). More provocative are the results of Ellis (1977) and Deitmer and Ellis (1978) obtained with sodium-sensitive microelectrodes. They reported that concentrations of cardiac glycosides between 10⁻⁸ and 10⁻⁷ M sometimes produce a small decrease in intracellular sodium. If inotropic
effects of ouabain can occur when myocardial [Na⁺] is diminished and are sodium dependent as suggested by the results in sodium-free solution, one of two conclusions can be reached: either glycosides produce a sodium-dependent inotropic action not dependent on sodium pump inhibition, or glycosides produce some effect other than sodium pump inhibition which tends to decrease [Na⁺].

Cardiac glycosides may decrease [Ca²⁺], by virtue of their ability to increase the release of endogenous norepinephrine (mentioned above). Agents which elevate cyclic AMP in cardiac muscle are known to stimulate calcium pumping into the sarcoplasmic reticulum (Kirchberger et al, 1974) and would be expected to reduce [Ca²⁺] (and, thus, [Na⁺]], via Na⁺-Ca²⁺ exchange) in quiescent preparations. Low concentrations of ouabain (5.5 x 10⁻⁵ M) have been reported to cause a substantial (>50%) acute increase in the output of endogenous norepinephrine from isolated guinea pig hearts (Harvey, 1975).

We can conclude that the inotropic effects of ouabain which are associated with sodium pump inhibition are, as expected, sodium dependent. Other hypothetical inotropic actions by ouabain, whether they be direct or indirect, also are sodium dependent.

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Cardiac architecture is such that the two pumps do not work independently of one another but interfere with each other's function. It has been shown, for instance, that the diastolic pressure volume relationship of one ventricle is dependent on the degree of filling of the other, a phenomenon which is even more pronounced at higher diastolic volumes if the pericardium is left intact (Elzinga, 1972; Elzinga et al., 1974; Bemis et al., 1974; Santamore et al., 1976; Glantz and Parmley, 1978; Glantz et al., 1978).

The intention of this study was to compare pump function and work output of the left and right ventricles and to examine the mutual interference of the two pumps during systole. We have restricted ourselves to the latter period because the diastolic...
Sodium requirement for effects of ouabain on contraction of isolated guinea pig atria.
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